

HYGROTHERMO CONDITIONED EXTRACTION OF NEMATODES FROM DRY FARMYARD MANURE

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A new method called hygrothermo-conditioned extraction (HTC extraction) of nematodes from dry farmyard manure is described to recover nematodes in dry farmyard manure. Moisture contents of a sample of manure was adjusted to 20% (w/v) followed by 72 hr incubation at 30°. After this, sub-samples consisting of 50 cm³ of conditioned manure were subjected to Baermann funnel extraction. By this method 49 nematodes – *Cephalobus litoralis*, *Helicotylenchus indicus*, *Eudorylaimus* sp. and *Psilenchus hilarus* in different life stages were recovered. In control 21 nematodes belonging to *C. litoralis* and *Eudorylaimus* sp. were recovered. Apparently *H. indicus* and *P. hilarus* survived in dry farmyard manure in anhydrobiotic state. They were recovered due to the pre-extraction conditioning of the manure through incubation under optimized moisture and temperature.

Key words: Nematode extraction, Methodology, Farmyard manure.

INTRODUCTION

Nematode dissemination through infested plants, soil, irrigation water etc. is frequently stressed but the role of farmyard manure is generally ignored. This probably emanates from the predominant belief that nematodes can survive only in thoroughly moist conditions. There seems to be too little awareness that many species of nematodes survive by cryptobiosis – the state of prolonged biological inactivity which enables organisms to survive exceedingly long periods of time in extremely unfavourable conditions of moisture, temperature, oxygen or osmotic pressure.

Farmyard manure often harbours many kinds of nematodes and when it dries out slowly, conditions are excellent for nematodes to enter into anhydrobiosis which is a form of cryptobiosis induced by insufficient moisture. Farmyard manure is an exportable commodity, and therefore, should be checked before export/import to prevent pathogenic nematodes from crossing the international frontiers. The conventional methods used for extraction neither reveal all the forms of nematodes nor their actual numbers present in the dry farmyard manure. This paper reports a method for detecting nematodes in dry farmyard manure by pre-extraction conditioning through incubation under optimized moisture and temperature.

MATERIALS AND METHODS

Dry farmyard manure was collected from a heap in the nursery of the Aga Khan Medical University, Stadium Road, Karachi, Pakistan. It was battered gently and passed

through 50 mesh sieve. The moisture content of the manure was determined by weighing a portion of it, drying it in an oven for 24 hr. and reweighing. 300 cm³ of the material was then measured in a glass cylinder by tapping the cylinder on the bench 10 times to settle the manure. Distilled water was added to bring the proportion of water to 20% (w/v). The cylinder was covered tightly with a piece of polyethylene sheet secured by a rubber band. The cylinder was agitated rigorously to distribute the water in the manure and placed in an incubator at 30° for 72 hr. The sample was then agitated again and divided equally into six sub-samples, each of which was placed on Baermann funnel for 48 hr. Nematodes were recovered in 20 ml aliquots. This hydration and incubation method is called hygrothermo-conditioned extraction (HTC extraction). A control set was placed on Baermann funnel but was not pre-conditioned. All treatments were replicated six times.

RESULTS AND DISCUSSIONS

With the HTC extraction method, an average of 49 nematodes per 50 cm³ of manure were recovered which included *Cephalobus litoralis* (Akhtar) (Andrassy, 1984), *Helicotylenchus indicus* Siddiqi, (1963), *Eudorylaimus* sp. (Andrassy, 1959), and *Psilenchus hilarus* Siddiqi, 1963. The control contained 21 nematodes probably *C. litoralis* and *Eudorylaimus* sp. (Table 1). In both treatments, second stage juveniles occurred, some with, and some without stylets. In Pakistan, the juveniles were in the same genera and species as mentioned above and might have emerged from eggs during conditioning and extrac-

Table 1. Nematodes recovered by hygrothermo extraction and ordinary extraction by Baermann funnel.

Nematode species	Number of nematodes recovered	
	HT extraction	Ordinary extraction
<i>Cephalobus litoralis</i>	9	7
<i>Eudorylaimus</i> sp.	7	5
<i>Helicotylenchus indicus</i>	5	0
<i>Psilenchus hilarus</i>	4	0
Stylet-bearing juveniles	9	2
Non stylet-bearing juveniles	15	7
Total:	49	21

tion. It is possible that adult females of *C. litoralis* recovered in the experiment developed from eggs already present in the manure since its life cycle is 3-4 days [1] and since it feeds on decaying organic matter. However, females of *H. indicus*, *Eudorylaimus* sp. and *P. hilarus* from the incubated sample almost surely survived in the dry manure as adults. *Helicotylenchus manni*, a close relative of *H. indicus*, is an anhydrobiotic nematode [2-4] as are *Eudorylaimus* sp. [5] and *P. hilarulus*, a close relative of *P. hilarus* [6]. Ferris [7] shares our view that moistening dry soil before extraction causes nematodes to emerge from a cryptobiotic state.

Improvement in the recovery of some nematodes by pre-process moistening of the soil has been reported earlier

[8]. Recently Ferris [7] stressed the necessity of moistening dry soil samples before extraction of nematodes and also gave a 12-point plan to promote extraction efficiency and consistency. However, no protocol for extracting nematodes from dry farmyard manure has hitherto been available. Our paper demonstrates the presence of nematodes in dry farmyard manure, and describes a method for their extraction. These results may be of great interest of other workers in the subject which may result in further improvement of methods for extracting nematodes from dry farmyard manure. This paper should also alert regulatory personnel to this method of nematode dissemination.

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